JACC Vol. 3, No. 5
May 1984:1252–5

Potentiation of Coronary Vascular Platelet Adhesion by Atrial Pacing in the Presence of Arterial Stenosis in Dogs

PAUL WALINSKY, MD, FACC, ALLAN M. LEFER, PhD, FACC, PETER FRASCA, MD, WILLIAM SANTAMORE, PhD

Philadelphia, Pennsylvania

The effect of atrial pacing on coronary hemodynamics and platelet adhesion was evaluated in 13 dogs. In all 13 dogs, a snare was placed around the circumflex artery and tightened so that distal coronary artery pressure decreased to 60 to 70 mm Hg. In 10 dogs, atrial pacing was instituted at twice the heart rate at rest for 10 minutes. In three dogs, observation was continued for 10 minutes without pacing. In the 10 dogs undergoing pacing, heart rate increased from 90.5 ± 32.6 to 173.5 ± 45.8 beats/min. Aortic pressure was unchanged. Distal coronary artery pressure decreased from 70.8 ± 7.8 to 53.2 ± 10.0 mm Hg ($p < 0.05$) and the pressure gradient across the stenosis increased from 47.6 ± 12.7 to 61.2 ± 9.1 mm Hg ($p < 0.05$). Stenotic resistance increased from 2.5 ± 0.8 to 3.6 ± 2.4 mm Hg/ml · min^{-1}, but coronary flow was unchanged. In all three control dogs, there was no change in coronary dynamics for the 10 minute period.

In 8 of the 10 dogs that underwent pacing, platelet deposition was observed at the site of coronary stenosis. In contrast, in the three control dogs there was no platelet deposition. Atrial pacing in the presence of coronary stenosis appears to alter coronary hemodynamics such that there are activation and deposition of platelets at the site of stenosis. This platelet deposition may be transient or could become the nidus for subsequent platelet-related events in the coronary vessel.

Platelets have been thought to be involved in coronary artery disease (1). However, a specific role for and specific mechanisms of platelet involvement in the pathophysiology of coronary artery disease remain undetermined. Folts et al. (2) demonstrated the development of platelet plugs in the presence of mechanical partial obstruction of a dog coronary artery. These platelet plugs occurred spontaneously after institution of an external compressive coronary artery stenosis. Their study suggests that there may be a clinical counterpart to the phenomenon of spontaneous platelet adhesion or activation. Undoubtedly, there are many factors that interact and enhance or inhibit platelet adhesion or aggregation in blood vessels.

We previously demonstrated an efflux of thromboxane B_2 from the coronary sinus in patients with significant coronary artery disease after atrial pacing (3). Mehta et al. (4) demonstrated decreased platelet count and decreased platelet aggregability of coronary sinus blood in patients with coronary artery disease. Platelet aggregation was increased by the stress of atrial pacing. Their findings suggest that platelet activation or adhesion occurred as a result of the stress of atrial pacing in patients with significant coronary artery disease. This type of platelet adhesion and activation could occur at a variety of sites, including the stenotic area and the microvasculature. Platelet adhesion could also be related to changes in the ischemic myocardium. The major purpose of our study was to test the hypothesis that platelet adhesion can be induced or enhanced at the site of a coronary stenosis by atrial pacing. This direct demonstration of platelet involvement is of value in understanding the potential role of platelets in coronary artery disease.

Methods

Experimental preparation. Thirteen mongrel dogs weighing between 28 and 36 kg were pretreated with morphine, 1 mg/kg body weight, and anesthetized with alpha-chloralose, 1 mg/kg, and mechanically ventilated with intermittent positive pressure ventilation. Supplemental doses of alpha-chloralose were given as needed to maintain anesthesia. Arterial partial pressure of oxygen (Po_2), partial pres-

From the Departments of Medicine and Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania. This study was supported by a research grant from the W.W. Smith Charitable Trust, Rosemont, Pennsylvania. Manuscript received May 31, 1983; revised manuscript received October 17, 1983, accepted November 28, 1983.

Address for reprints: Paul Walinsky, MD, Department of Medicine, Jefferson Medical College, 11th and Chestnut Streets, Philadelphia, Pennsylvania 19107.

0735-1097/84/$3.00

sure of carbon dioxide (P_{CO_2}) and pH were periodically monitored throughout the experiment using an Instruments Laboratory model 113 blood gas analyzer. Respiratory adjustments or intravenous infusion of sodium bicarbonate were instituted when necessary to maintain appropriate P_{O_2}, P_{CO_2} and pH. The heart was exposed through a left lateral thoracotomy and suspended in a pericardial cradle. A catheter was advanced in retrograde manner from the right femoral artery and positioned in the ascending aorta to measure aortic pressure. A long section of the left circumflex artery was isolated. A 2.5 mm electromagnetic flow probe (In Vivo Metric Systems) was placed proximally around the coronary artery. A silk suture was placed distal to the flow probe. A marginal branch distal to the suture was isolated and a 22 gauge, 1 inch (2.54 cm) catheter (Angiocath, Deseret Pharmaceuticals Inc.) was inserted to monitor distal coronary artery pressure.

Coronary stenosis and atrial pacing. The right internal jugular vein was isolated and a pacing electrode was positioned in the right atrium. The suture around the coronary artery was attached through rigid polyethylene tubing to a machinist's micrometer. The snare was gradually tightened until the distal coronary artery pressure was decreased to 60 to 70 mm Hg. This corresponded to a stenosis of 90% or greater. After the demonstration of stability over a 5 to 10 minute period, atrial pacing at a rate twice that of baseline heart rate was initiated and continued for 10 minutes. In the three control dogs, atrial pacing was not performed and the animals were observed an additional 10 minutes before excision of the artery. The total procedure required 15 to 20 minutes for completion.

Electron microscopic studies. After the 10 minutes of pacing, a 2 to 3 cm section of coronary artery with the stenosed site in the center was surgically excised and placed in 2.5% glutaraldehyde in 0.1 *M* phosphate buffer at 4°C for 2 hours. The vessel was then bisected and washed twice in 0.1 *M* phosphate buffer. Postfixation was carried out for 1.5 hours in 1% aqueous osmium textroxide (OsO_4) at 4°C, after which the tissue was rinsed three times in buffer. The tissue samples were dehydrated in a graded series of acetone solutions. These samples were then critical point dried and placed on aluminum specimen mounts with silver tape. A thin coat of gold was deposited on the samples in a Polaron sputter coater, and the tissue was then examined in a JEOL model 35C scanning electron microscope at a variety of magnifications.

Statistical analysis. Statistical evaluation was made with Student's *t* test. Values of probability (p) less than 0.05 or less were taken as the level of significance.

Results

Coronary dynamics. Ten dogs were subjected to cardiac pacing and three control dogs were exposed to all the surgical procedures but not to pacing. In the dogs undergoing pacing, heart rate increased from 90.5 ± 32.6 to 173.5 ± 45.8 beats/min ($p < 0.005$) (Table 1). However, there was no significant change in aortic pressure even at the peak of the heart rate increase. Coronary artery pressure could not be measured in one dog and coronary flow could not be measured in three additional dogs. Mean coronary pressure distal to the stenosis decreased from 70.8 ± 7.8 to 53.2 ± 10.0 mm Hg ($p < 0.05$), whereas the mean pressure gradient across the stenosis increased from 47.6 ± 12.7 to 61.2 ± 9.0 mm Hg ($p < 0.05$). There was no change in coronary flow (23.4 ± 11.4 to 23.8 ± 12.3 cc/min). The stenotic resistance increased from 2.5 ± 0.8 to 3.6 ± 2.4 mm Hg/ml · min^{-1}.

Platelet deposition after pacing. Figure 1 demonstrates a scanning electron micrograph at the site of stenosis ob-

Figure 1. Scanning electron micrograph of a control dog coronary artery at the site of stenosis. Pacing was not performed in this dog. There is no evidence of abnormal platelet deposition. **Upper panel** × 1,000, **lower panel** × 3,000; both reduced by 9%.

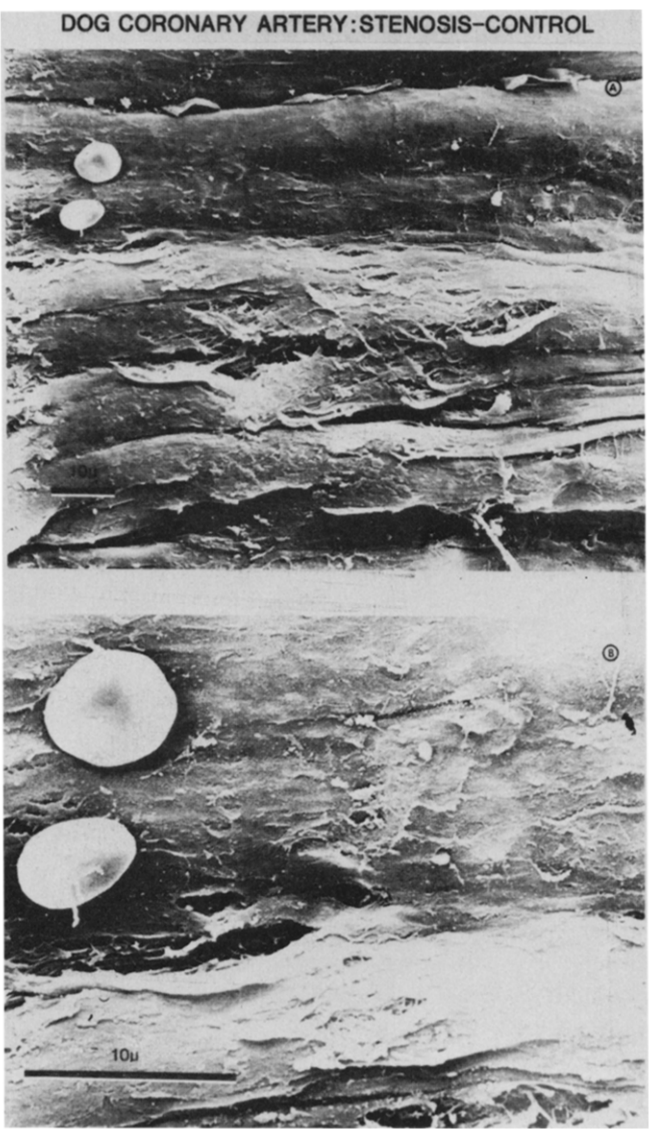


Table 1. Coronary Hydraulic Variables Before and After Atrial Pacing

	Heart Rate (beats/min)	Aortic Pressure (AoP) (mm Hg)	Distal Coronary Pressure (DCP) (mm Hg)	Gradient AoP-DCP (mm Hg)	Coronary Flow (ml/min)	Stenotic Resistance (mm Hg/ml · min^{-1})	Distal Coronary Resistance (mm Hg/ml · min^{-1})
Before pacing	90.5 ± 32.6	118.6 ± 14.4	70.8 ± 7.8	47.6 ± 12.7	23.4 ± 11.4	2.5 ± 0.8	3.5 ± 1.7
During pacing	173.5 ± 45.8†	116.3 ± 12.7	53.2 ± 10.0*	61.2 ± 9.1*	23.8 ± 12.3	3.6 ± 2.4	3.3 ± 2.2

*$p < 0.05$; †$p < 0.005$. All values are mean ± standard deviation.

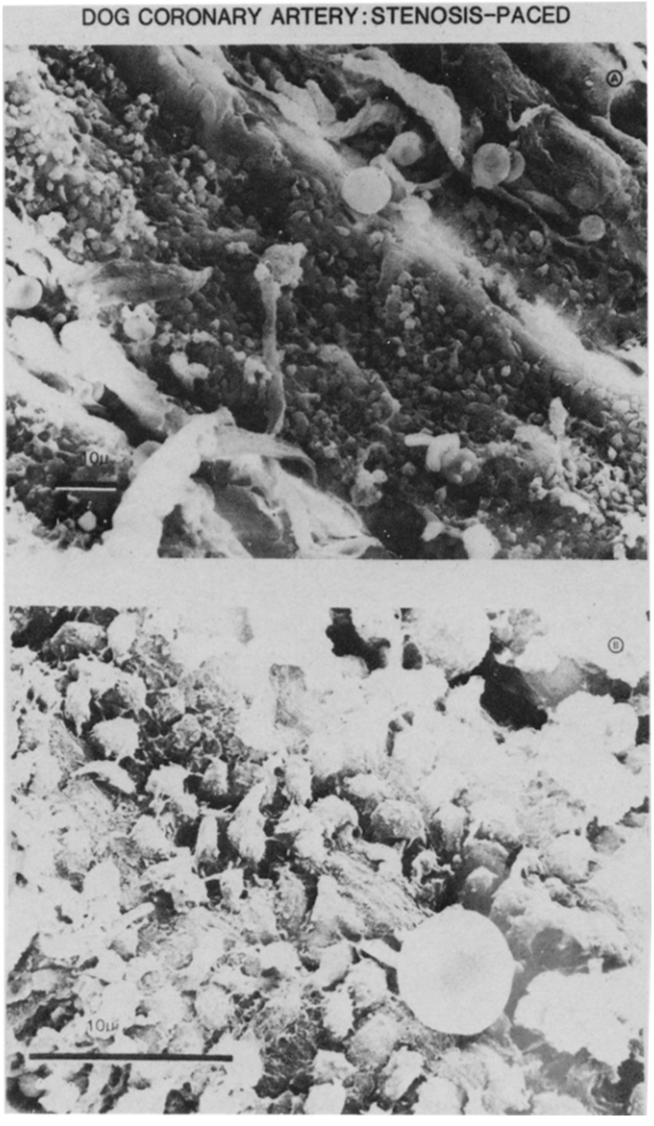
tained from a control dog. The endothelium appears relatively normal and there is no evidence of platelet adhesion. Figure 2 shows a scanning electron micrograph taken from the coronary artery at the site of stenosis in a dog that underwent pacing. The endothelial surface of the artery is covered with marked platelet deposition with large clusters of platelets. Eight of the 10 dogs that underwent pacing demonstrated such platelet deposition. No platelet deposition was noted in any of the control dogs.

Discussion

Platelet aggregates have been demonstrated in stenotic coronary vessels in experimental animal studies and in human pathologic studies. Such platelet aggregates have been seen in the large epicardial vessels as well as in the microvasculature (5,6). The development of platelet adhesion and subsequent aggregation at the site of stenosis could lead to an unstable clinical syndrome if periodic occlusion of a vessel or increased severity of a stenosis occurred. If platelet aggregates were washed out from a large vessel stenotic site and caused either mechanical occlusion of distal smaller vessels or release of vasoactive substances that could induce distal vasospasm, an unstable clinical syndrome could likewise be produced.

Cause of platelet deposition. In our study following the institution of a high grade stenosis, platelet deposition was seen in 8 of 10 animals after a pacing-induced increase in heart rate. Such platelet deposition was not seen in the dogs that had a similar underlying stenosis but did not undergo pacing. Changes in coronary flow dynamics induced by atrial pacing included an increased pressure gradient across the stenosis, an increased calculated stenotic resistance and a decreased diastolic flow time across the stenosis with a presumed increased velocity of flow across the stenosis. It is likely that the platelet deposition was related to alteration of platelet function caused by altered coronary flow dynamics and consequent increased blood flow turbulence and platelet shear stress (7–9). Changes in endothelial integrity were not evaluated and, therefore, damage to the endothelium may not be ruled out as a factor in the platelet deposition. However, endothelial damage would have been expected to be similar in the paced and unpaced animals and cannot, alone, explain the observed differences between the two groups. The previous observation (3) of thromboxane B_2 efflux from the coronary sinus after atrial pacing in pa-

Figure 2. Scanning electron micrograph of a dog coronary artery at the site of stenosis after 10 minutes of atrial pacing. Note the deposition of sheets of platelets. **Upper panel** × 1,000, **lower panel** × 3,000; both reduced by 9%.



tients with severe coronary artery disease but not in patients with normal coronary arteries is consistent with the concept of platelet activation induced by changes in coronary flow dynamics caused by tachycardia in the presence of significant stenosis.

Folts et al. (2) demonstrated that occlusive events at a stenosis may be mediated by platelets. Our study used a different type and shorter time course of partial vessel occlusion. The platelet involvement demonstrated by Folts et al. resulted in total occlusion and occurred after longer periods of observation. However, lesser degrees of obstruction ranging from sparse adhesion to the sheets of platelets seen in the current study must precede the evolution to complete occlusion.

Effect of beta-adrenergic blocking agents. This study demonstrates that an increase in myocardial oxygen demand subsequent to tachycardia that produces an imbalance in oxygen supply and demand may also lead to intraluminal platelet adhesion at a stenotic site. Thus, agents such as beta-adrenergic blockers, which decrease myocardial oxygen demand, may also inhibit the hemodynamic events leading to platelet adhesion across a stenotic vascular bed. Indeed, it has been suggested (10) that beta-adrenergic blocking agents may beneficially affect platelet function in patients with coronary artery disease. It is not clear whether alterations of flow characteristics relate to these changes, but the data obtained in our study are consistent with this concept.

Implications. We have demonstrated that alteration of coronary flow dynamics caused by atrial pacing results in significant adhesion of platelets to the wall of the stenosed coronary artery. This sequence of events could be of clinical significance by becoming the initiating focus of platelet-related events in the coronary vessel.

We gratefully acknowledge the technical assistance of Matt McVey and David Kreitzer during the course of these studies.

References

1. Turpie AGG, deBoer AC, Genton E. Platelet consumption in cardiovascular disease. Semin Thromb Hemost 1982; 8:161–85.
2. Folts JD, Crowell EB, Rowe GG. Platelet aggregation in partially obstructed vessels and their elimination with aspirin. Circulation 1976; 54:365–70.
3. Lewy RI, Wiener L, Walinsky P, Lefer AM, Silver MJ, Smith JB. Thromboxane release during pacing induced angina pectoris: possible vasoconstrictor influence on the coronary vasculature. Circulation 1980; 61:1165–71.
4. Mehta J, Mehta P, Pepine CJ. Platelet aggregation in aortic and coronary venous blood in patients with and without coronary disease. Role of tachycardia stress and propranolol. Circulation 1978;58:881–6.
5. Haerem JW. Sudden coronary death: the occurrence of platelet aggregates in the epicardial arteries of man. Atherosclerosis 1971;14:417–32.
6. Haerem JW. Platelet aggregates in intramyocardial vessels of patients dying suddenly and unexpectedly of coronary artery disease. Atherosclerosis 1972;15:199–213.
7. Brown CH III, Leverett LB, Lewis CW, Alfrey CP Jr. Hellums JD. Morphological, biochemical, and functional changes in human platelets subjected to shear stress. J Lab Clin Med 1975;86:462–71.
8. Turrito VT, Baumgartner HR. Platelet interaction with subendothelium in flowing rabbit blood: effect of blood shear rate. Microvasc Res 1979;17:38–54.
9. Gould KL. Dynamic coronary stenosis. Am J Cardiol 1980;45:286–92.
10. Weksler BB, Gillick M, Pink J. Effect of propranolol on platelet function. Blood 1977;49:185–96.